Neuron
Previews

CellPress

A neuropeptide regulates immunity across species

Liwen Deng[1] and Isaac M. Chiu[1,*]
[1]Department of Immunology, Harvard Medical School, Boston, MA 02115, USA
*Correspondence: isaac_chiu@hms.harvard.edu
https://doi.org/10.1016/j.neuron.2022.03.036

Communication between the nervous system and immune system is important for regulating immunity in health and disease. Yu et al. (2022) show that neuropeptide Y and its homolog NPF serve as a "language" to facilitate crosstalk between these two systems across species, enabling neurons to downregulate harmful immune responses.

The nervous and immune systems are complex systems that function in peripheral tissues to coordinate physiology. Increasing evidence points to key roles for neuroimmune communication in organismic homeostasis and inflammation. Immune cells express neurotransmitter and neuropeptide receptors, allowing them to respond to sensory and autonomic neurons (Udit et al., 2022). The spleen is an immune organ that produces cytokines including TNF-α and is innervated by post-ganglionic neurons whose cell bodies reside in the suprarenal and celiac ganglia (SrG-CG). Vagus nerve stimulation (VNS) protects against lipopolysaccharide (LPS)-induced shock through splenic immunity (Borovikova et al., 2000, Huston et al., 2006). Splenic nerves release norepinephrine (NE), which acts on T cells to produce acetylcholine, which then suppresses macrophage production of TNF-α (Rosas-Ballina et al., 2011). While these studies show roles for neurotransmitters in the spleen, how neuropeptides influence splenic immunity is less well understood.

Neuropeptide Y (NPY) is a 36-amino-acid neuropeptide that has roles in regulating food intake, neurogenesis, stress, vasoconstriction, and immunomodulation (Zukowska et al., 2003, Shende and Desai, 2020, Liu et al., 2020). In the peripheral nervous system, NPY is mainly expressed by sympathetic neurons and is released during stress (Shende and Desai, 2020). The physiological role of NPY in host defense is not well understood. In this issue of *Neuron*, Yu et al. (2022) utilize approaches from neurobiology, microbiology, and immunology to reveal a role for NPY and its homolog neuropeptide F (NPF) in regulating splenic and innate immunity across species (rats, *Drosophila*, mice, humans).

Yu et al. began their study by determining how SrG-CG neurons sense and modulate splenic immune responses (Figure 1A). In a rat model of LPS-induced systemic inflammation, they performed RNA-sequencing of SrG-CG to identify gene signatures responding to inflammation. LPS treatment induced marked upregulation of *Npy* in SrG-CG, while expression of other neuropeptides was unaffected. They next performed calcium imaging, finding that SrG-CG neurons can be activated by LPS *in vitro* to induce calcium influx, indicating that these neurons can directly sense this microbial signal. Furthermore, the LPS receptor Toll-like receptor 4 (*Tlr4*) was expressed by SrG-CG neurons. Pseudorabies-virus-mediated retrograde tracing showed that these NPY^{+} neurons originated in the SrG-CG.

To investigate how NPY regulates splenic immune responses, the authors next knocked down *Npy* in SrG-CG by RNA interference (RNAi) and treated rats with LPS. Flow cytometry and single-cell RNA-sequencing found that loss of NPY reduced numbers of splenic T cells. B cells, T cells, and monocytes responded to *Npy* interference by downregulating genes involved in suppression of immune functions. Transcriptional profiling showed that Npy receptor *Npy1r* was expressed in T cells and macrophages. Accordingly, treatment with exogenous NPY had no effect on B cells, while T cells and macrophages responded by significant reductions in expression of inflammatory cytokines. NPY treatment also inhibited LPS-induced cytokine secretion by cultured splenocytes. Using splenocyte/SrG-CG neuron co-cultures, the authors found that *Npy* knockdown in neurons led to higher LPS-induced upregulation of *Tnf-α*, *Il-6*, *Il-1β*, and *Ccl-2* compared to control siRNA. *In vivo*, silencing *Npy* expression in SrG-CG neurons prior to LPS treatment resulted in higher levels of *Tnf-α*, *Il-6*, *Il-1β*, and *Ccl-2* in spleens, whereas overexpression of *Npy* decreased these cytokines.

Given their findings that SrG-CG neurons respond to LPS and that neuronal NPY downregulates splenic immune responses, the authors hypothesized that NPY may limit inflammation during bacterial infection. In an *E. coli* infection model, SrG-CG *Npy*-knockdown rats exhibited decreased survival compared to control rats, and SrG-CG *Npy*-overexpressing rats had higher survival. Analysis of spleens from *E.-coli*-infected rats revealed more tissue damage and hemorrhaging and higher TNF-α levels in the *Npy*-knockdown group. Splenic denervation abolished differences in splenic TNF-α levels between control and *Npy*-knockdown rats. Treatment of rats with the NPY1R antagonist BIBP3226 resulted in higher *Tnf-α*, *Il-6*, and *Il-1β* levels in response to LPS, indicating a role for this NPY receptor in immunity.

The authors next examined whether this mechanism extended beyond mammals. They investigated the role of NPF, a homolog of NPY expressed by *Drosophila melanogaster* (Brown et al., 1999), in bacterial infections by knocking down *NPF* using a neuron-specific RNAi approach (Figure 1B). During acute infection with the gram-negative pathogen Ecc15, neuronal NPF^{RNAi} flies had higher expression of the antimicrobial peptides *cecropin* and *drosocin* and cytokines *eiger* and *spaetzle* compared to that of control flies. The authors recovered less bacteria from NPF^{RNAi} flies compared to

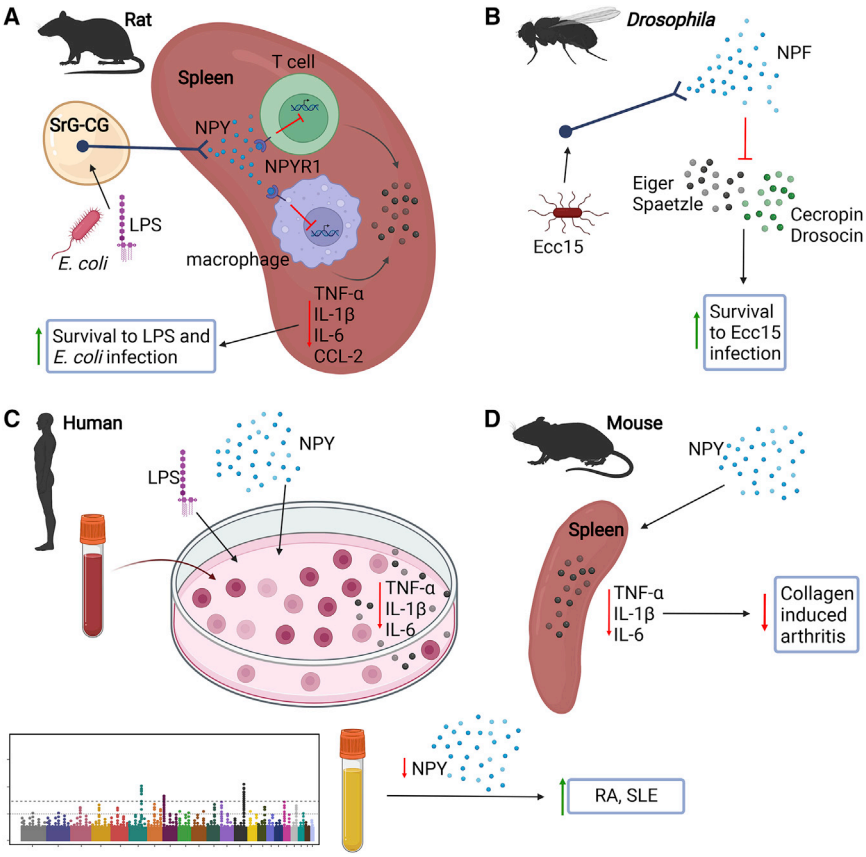

Figure 1. NPY and NPF regulate immune responses in different species

(A) Rat SrG-CG neurons release NPY in the spleen that acts through NPYR1 to downregulate T cell and macrophage cytokine expression in response to LPS treatment and *E. coli* infection.

(B) *Drosophila* NPF signaling decreases cytokine and antimicrobial peptide expression during Ecc15 infection, improving survival.

(C) Human blood leukocytes decrease cytokine expression when co-treated with LPS and NPY. *NPY* SNPs and lower serum NPY are linked to SLE and RA.

(D) Mouse NPY reduces inflammatory cytokines in the spleen during collagen-induced arthritis.

Created with BioRender.com. SrG-CG, suprarenal and celiac ganglia; NPY, neuropeptide Y; NPYR1, neuropeptide Y receptor 1; LPS, lipopolysaccharide; NPF, neuropeptide F; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

controls. NPF^{RNAi} flies exhibited higher mortality, while knocking out *eiger* in NPF^{RNAi} flies increased survival. These results show that upregulation of immune response can be detrimental during acute bacterial infection and that NPF/NPY may have an evolutionarily conserved role in limiting inflammation.

Finally, the authors were interested to know if NPY was relevant to human disease (Figure 1C). Examining human genome-wide association studies (GWAS) data, they identified single-nucleotide polymorphisms (SNPs) near *NPY* and its receptor loci that were correlated with autoimmune diseases. Comparing serum NPY levels in samples from autoimmune disease patients to those of healthy controls, NPY was significantly lower in patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). In cultured human blood lymphocytes, NPY inhibited expression of *TNF-α*, *IL-6*, and *IL-1β* in response to LPS treatment. Using a collagen-induced arthritis model, the authors showed that *Npy* knockdown resulted in higher splenic *Tnf-α*, *Il-6*, and *Il-1β* levels and increased arthritis disease severity in mice (Figure 1D).

Several key questions remain for future study. How do NPY^{+} neurons sense microbial infections? While the authors detect *Tlr4* expression in SrG-CG neurons, the functional relevance of this receptor in neuronal bacterial detection remains to be determined. Do vagal sensory or somatosensory neurons signal to SrG-CG neurons to induce NPY release? A recent study found that electrical stimulation of abdominal sensory nerves induces NPY^{+} SrG-CG neuron activation and suppresses LPS inflammation (Liu et al., 2020). The vagus nerve also signals via spleen-innervating neurons to regulate LPS inflammation (Borovikova et al., 2000). In these circuits, NE acts through β2-adrenergic receptors on splenic immune cells to suppress cytokine production (Liu et al., 2020, Rosas-Ballina et al., 2011). Does NPY signaling synergize with NE and other neurotransmitters to modulate immune cell phenotypes? Future work is also needed to determine how NPY regulates adaptive or innate immune responses beyond infection in humans. For example, the mechanisms by which changes in NPY levels influence RA or SLE pathogenesis in humans remain to be defined.

The balance of immune responses is critical for homeostasis and responses to infection. Yu et al. reveal a role for neural signaling through NPY in tuning inflammation. They find that NPY/NPF represents a conserved language that neurons utilize to downregulate immune activation and cytokine expression and that disrupting this communication can have detrimental effects during bacterial infection and increase susceptibility to autoimmune disease.

ACKNOWLEDGMENTS

We acknowledge support from the NIH (R01DK127257 and 5R01AI130019), the Burroughs Wellcome Fund, and the Drako Family Fund for Allergy Research.

DECLARATION OF INTERESTS

I.M.C. serves on scientific advisory boards for GSK Pharmaceuticals and LIMM Therapeutics, and his lab receives funding from Allergan/Abbvie Pharmaceuticals.

REFERENCES

Borovikova, L.V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G.I., Watkins, L.R., Wang, H., Abumrad, N., Eaton, J.W., and Tracey, K.J. (2000). Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature *405*, 458–462.

Brown, M.R., Crim, J.W., Arata, R.C., Cai, H.N., Chun, C., and Shen, P. (1999). Identification of a

Drosophila brain-gut peptide related to the neuropeptide Y family. Peptides *20*, 1035–1042.

Huston, J.M., Ochani, M., Rosas-Ballina, M., Liao, H., Ochani, K., Pavlov, V.A., Gallowitsch-Puerta, M., Ashok, M., Czura, C.J., Foxwell, B., et al. (2006). Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. J. Exp. Med. *203*, 1623–1628.

Liu, S., Wang, Z.F., Su, Y.S., Ray, R.S., Jing, X.H., Wang, Y.Q., and Ma, Q. (2020). Somatotopic Organization and Intensity Dependence in Driving Distinct NPY-Expressing Sympathetic Pathways by Electroacupuncture. Neuron *108*, 436–450 e7.

Rosas-Ballina, M., Olofsson, P.S., Ochani, M., Valdés-Ferrer, S.I., Levine, Y.A., Reardon, C., Tusche, M.W., Pavlov, V.A., Andersson, U., Chavan, S., et al. (2011). Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. Science *334*, 98–101.

Shende, P., and Desai, D. (2020). Physiological and Therapeutic Roles of Neuropeptide Y on Biological Functions. Adv. Exp. Med. Biol. *1237*, 37–47.

Udit, S., Blake, K., and Chiu, I.M. (2022). Somatosensory and autonomic neuronal regulation of the immune response. Nat. Rev. Neurosci. *23*, 157–171.

Yu, J., Xiao, K., Chen, X., Deng, L., Zhang, L., Li, Y., Gao, A., Gao, J., Wu, C., Yang, X., et al. (2022). Neuron-derived neuropeptide Y fine-tunes the splenic immune responses. Neuron *110*, 1327–1339.

Zukowska, Z., Pons, J., Lee, E.W., and Li, L. (2003). Neuropeptide Y: a new mediator linking sympathetic nerves, blood vessels and immune system? Can. J. Physiol. Pharmacol. *81*, 89–94.

CLP1-dependent premature transcription termination opposes neurodegeneration

Michal R. Gdula,[1,2] Magda Kopczyńska,[1,3] Upasana Saha,[1,3] and Kinga Kamieniarz-Gdula[1,3,*]
[1]Center for Advanced Technology, Adam Mickiewicz University, Uniwersytetu Poznanskiego 10, 61-614 Poznań, Poland
[2]Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland
[3]Department of Molecular and Cellular Biology, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland
*Correspondence: kinga.kamieniarz-gdula@amu.edu.pl
https://doi.org/10.1016/j.neuron.2022.03.012

Usage of alternative mRNA 3′ ends has profound functional consequences, particularly in the nervous system. In this issue of *Neuron*, LaForce et al. (2022) dissect the effect of CLP1 on mRNA 3′ end diversity in motor neuron models of neurodegeneration.

Pontocerebellar hypoplasia type 10 (PCH10) is a rare neurodegenerative disorder affecting children. PCH10 patients have severe intellectual disability, motor dysfunction, seizures, and microcephaly. In 2014 the Gleeson and Lupski labs independently identified the genetic basis for PCH10: homozygosity for the R140H (arginine to histidine) missense mutation in CLP1 (cleavage factor polyribonucleotide kinase subunit 1) (Weitzer et al., 2015). CLP1 is a component of the pre-mRNA 3′ end-processing machinery and also associated with the tRNA splicing endonuclease complex. The original studies describing CLP1 R140H involvement in PCH10 concentrated on its effect on tRNA processing. Indeed, multiple neurological disorders, including several forms of PCH, result from abnormal tRNA biogenesis. However, in the present study, LaForce et al. (2022) sought to understand the role of CLP1 and its R140H mutation in the regulation of mRNA 3′ isoform diversity. mRNA 3′ isoforms arise as a result of alternative usage of polyadenylation sites (PASs) in a phenomenon called alternative polyadenylation (APA). APA leads to, e.g., altered mRNA stability or localization to subcellular compartments, which impacts cellular functions (Bae and Miura, 2020).

The authors employed two human cell culture models. In the main model, induced pluripotent stem cells (iPSCs) were derived from fibroblasts of an unaffected parent (heterozygous CLP1 R140H carrier) and affected PCH10 child (homozygous for CLP1 R140H) and subsequently differentiated to motor neurons (Figure 1A). In another model, H9 human embryonic stem cells were gene edited using CRISPR-Cas9 to generate a CLP1 knockout cell line ($CLP1^{KO}$), which was then also differentiated to motor neurons in parallel to a wild-type cell line ($CLP1^{WT}$). $CLP1^{KO}$ motor neuron cultures differentiated at a lower density than $CLP1^{WT}$ controls but were morphologically indistinct and expressed the expected motor-neuron-specific factors. Similarly, the iPSC-derived motor neuron culture from the affected child showed lower density, but the cells were otherwise indistinct from the unaffected parent. To investigate the cause of reduced cell density in $CLP1^{KO}$ and affected CLP1 R140H mutant motor neuron cultures, the authors performed immunostaining for markers of motor neurons (ISL1/2) and motor neuron progenitors (OLIG2) from day 22 to day 28 of differentiation, assessing proliferation by co-staining with p-histone H3 and determining cell death by cleaved caspase 3. $CLP1^{KO}$ had a reduced proportion of proliferating motor neuron progenitors and showed increased apoptosis of both motor neurons and their progenitors when